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Genomic and Strontium Isotope Variation Reveal Immigration Patterns in a Viking Age Town

Highlights

- A case study reveals mobility between population clusters within the Viking world
- Archaeology, osteology, genetics, and isotopes highlight nuanced migration patterns
- Local, regional, and long-distance immigrants are identified in the town of Sigtuna
- We report 23 new ancient human genomes from the 10th to the 12th century CE Sweden

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In Brief

Krzewińska et al. report genetic and isotopic data from 23 Viking Age individuals from Sweden. By complementing archaeological and osteological data with paleogenetic and isotope data, a high level of mobility within the early medieval world can be detected, revealing the cosmopolitan nature of the late Viking towns.

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Genomic and Strontium Isotope Variation Reveal Immigration Patterns in a Viking Age Town

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SUMMARY

The impact of human mobility on the northern European urban populations during the Viking and Early Middle Ages and its repercussions in Scandinavia itself are still largely unexplored. Our study of the demographics in the final phase of the Viking era is the first comprehensive multidisciplinary investigation that includes genetics, isotopes, archaeology, and osteology on a larger scale. This early Christian dataset is particularly important as the earlier common pagan burial tradition during the Iron Age was cremation, hindering large-scale DNA analyses. We present genome-wide sequence data from 23 individuals from the 10th to 12th century Swedish town of Sigtuna. The data revealed high genetic diversity among the early urban residents. The observed variation exceeds the genetic diversity in distinct modern-day and Iron Age groups of central and northern Europe. Strontium isotope data suggest mixed local and non-local origin of the townspeople. Our results uncover the social system underlying the urbanization process of the Viking World of which mobility was an intricate part and was comparable between males and females. The inhabitants of Sigtuna were heterogeneous in their genetic affinities, probably reflecting both close and distant connections through an established network, confirming that early urbanization processes in northern Europe were driven by migration.

INTRODUCTION

The Early Medieval Period of northern Europe (ca. 8th–11th century) saw the formation of the Viking world with population growth and urbanization. Already at an early phase of this period, there was a notable population expansion from Scandinavia, not least with the Viking conquest and colonization. The urbanization process in Scandinavia started with the Viking proto-town Birka together with the contemporaneous Ribe, Hedeby, and Kaupang all focusing mainly on trade and craft production. Different forms of Christianity expanded northward, and the pagan ways were pushed back [1]. Goods, wealth, and people moved extensive geographic distances over established networks in northeastern Europe and the first stable urban centers emerged, including Kiev, Schleswig, York, Lund, and Sigtuna [2–4]. These became important hubs for the commercial and religious activity, as well as local and regional politics.

The early medieval period has been singled out as a period with high mobility, and the Viking expansion was an important part of this [5]. Expansion from Scandinavia was undertaken through seafaring often within the urban networks [6]. The mobility of the Vikings has, in fact, been considered one of the major forces shaping the demographic landscape of modern Europe [7–9]. Analysis of stable isotopes of individuals buried at Birka showed that some individuals were of non-local origin, as judged from $\delta^{34}S$ values [10]. This was recently confirmed by strontium (Sr) analyses of human remains [11, 12]. Furthermore, a number of genetic studies of individuals denoted as Vikings have been undertaken, but so far they have mainly focused on extant individuals or uniparental markers and single-nucleotide polymorphisms (SNPs) from Viking Age human remains [13–16], with only one full genome from Birka published thus far [11]. These studies revealed traces of uniparental genetic Viking legacy in the British Isles, the Orkneys, Iceland, and elsewhere [17]. Few genome-wide studies from Europe have been undertaken on human skeletal remains from the Viking Age. One reason for this is that during the Late Iron Age, the most common pagan burial practice was cremation (with inhumations as exceptions to the rule), leaving no material suitable for DNA analysis. However, early Christian contexts allow for larger genomic studies.

The town of Sigtuna in eastern central Sweden was one of the pioneer urban hubs in the vast and complex communicative

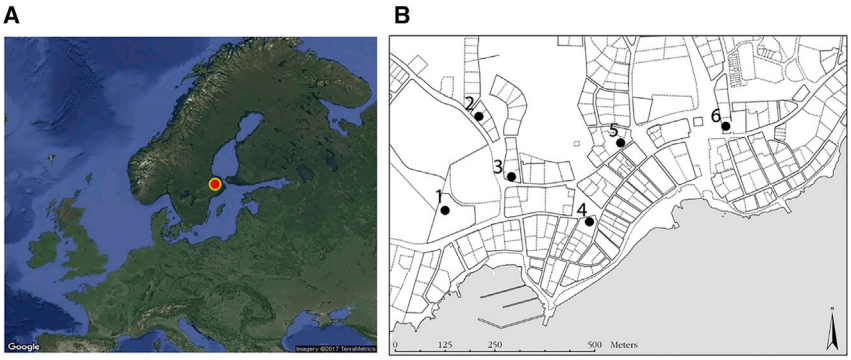

Figure 1. Sigtuna Location and the Site Organization

Map indicating location of Sigtuna (A), and town plan of Sigtuna with marked location of known medieval burial grounds (B) (adapted from Kjellström and Wikström [18]). 1, cemetery 1 (the Nunnan block); 2, cemetery 2 (the Kålsängen block); 3, cemetery 3 (the Kållandet block); 4, church 1 (St. Gertrud and the Urmakaren block); 5, the mass grave at St. Lars; 6, cemetery 4 (Bensinstationen and Götes Mack). See also Table S1.

network of the Viking world (Figure 1A). The town that is thought to have been royally founded was planned and organized as a formal administrative center and was an important focal point for the establishment of Christianity [19]. The material culture in Sigtuna indicates that the town had intense international contacts and hosted several cemeteries with a Christian character (Figure 1B; Tables S1 and S2). Some of them may have been used by kin-based groups or by people sharing the same sociocultural background. In order to explore the character and magnitude of mobility and migration in a late Viking Age town, we generated and analyzed genomic (n = 23) and strontium isotope (n = 31) data from individuals excavated in Sigtuna (Figure S1A; Tables S1 and S3). Strontium isotope ratio ($^{87}Sr/^{86}Sr$) is an established marker for mobility [20] and provides information on individual movement and life history in relation to geological isotopic signature of the landscape. Thus, strontium shows where a person lived (geographically) at the time of tissue (tooth enamel) formation, whereas genetics show to whom or to which group a person is related (in broad context). Given that strontium values present a finer scale of sub-structuring than genomics, we combine the two in order to investigate human mobility patterns in Sigtuna (Figures S1B and S1C).

Results and Discussion

We generated genome-wide data (0.01× to 3.7× genome coverage) from 23 individuals dated from the 10th to the 12th century CE (Tables 1 and S3). Whenever possible, the choice of the osteological material focused on dental material, and in the absence of teeth, compact long bones were chosen over other skeletal fragments such as mandibles or metacarpals (Figure S1A; Table S1). Among the sequenced individuals, 14 were genetically confirmed males and nine were females. Sequenced DNA fragments exhibited damage patterns typical of ancient DNA (Figure S2), contamination estimates using mitochondrial DNA (mtDNA) did not exceed 1% for any of the investigated samples, and the X-based contamination estimates in males were low (Table S3). We further analyzed teeth samples from 16 of these 23 individuals for strontium isotopes together with samples from teeth of additional 15 individuals. The 31 individuals represent six different burial contexts from the initial phase of the town's existence. The six contexts are four cemeteries, i.e., burial grounds without churches (cemetery 1, Nunnan; cemetery 2, Kålsängen; cemetery 3, Kållandet; and cemetery 4, Bensinstationen and/or Götes Mack); one mass grave (St. Lars); and one churchyard, i.e., a burial ground with an associated church (church 1, St. Gertrud and/or Urmakaren). The burial contexts most likely represent different groups of town dwellers. The graves and skeletons have previously been archaeologically documented and anthropologically analyzed [21–25].

The mitochondrial genomes were sequenced at 1.5× to 367× coverage. Most of the individuals were assigned to haplogroups commonly found in current-day Europeans, such as H, J, and U [14, 26, 27]. All of these haplotypes are present in Scandinavia today (Figure S3A).

The Y chromosome haplogroups were assigned in seven males. The Y haplogroups include I1a, I2a, N1a, G2a, and R1b (Table 1; Data S1, sheet 2). Two identified lineages (I2a and N1a) have not been found in modern-day Sweden or Norway [28, 29]. Haplogroups I and N are associated with eastern and central Europe, as well as Finno-Ugric groups [30]. Interestingly, I2a was previously identified in a middle Neolithic Swedish hunter-gatherer dating to ca. 3,000 years BCE [31].

The Genetic and Isotopic Diversity of Viking Age Sigtuna

In order to compare genetic relationships among the individuals from Sigtuna and modern-day and ancient individuals from Europe, we performed principal-component analysis (PCA) using a reference panel of 21 present-day European populations [32, 33] (Figure S3C). We projected all 23 ancient Sigtuna individuals together with Iron Age individuals from England (n = 10; from 360–350 calibrated [cal] BCE to 690–881 cal CE), Hungary (n = 1; 980–830 cal BCE), Montenegro (n = 1; dating not available) and Sweden (n = 1; 427–611 cal CE) [34–36] onto PC1 and PC2 of the modern-day individuals using Procrustes transformation [37] (Figure 2A). The Sigtuna individuals were widely distributed, with most falling within modern-day variation of Northern Europeans, with the majority of individuals encompassing genomic variation of modern-day individuals from Norway, the North Atlantic Islands, and England. Some samples show stronger tendencies toward modern Eastern, Western, and Central Europeans instead of Northern European populations (Figures 2A and 3). This broad distribution largely overlaps with the distribution of Iron Age English samples and most individuals cluster together with the single sample from Oxie in southern Sweden dated to the 4th–6th century CE. Thus, the PCA indicated substantial heterogeneity among all individuals from Sigtuna and even within sites (Figures S3B and S3C). This pattern also seems to be consistent for ten samples with coverages >0.25×, which are less affected by noise than those with lower coverage.

Table 1. Summary Statistics Based on Genome Sequence of 23 Individuals from Sigtuna

Sample ID	Location/Burial Site	Genome Coverage	mtDNA Genome Coverage	Mol. Sex	mtDNA Haplogroup	Y Chromosome Haplogroup
84001	cemetery 1 (Nunnan)	×3.7	×108.2	XY	H2a2a1g	N1a1a1a1a1 (N-L392*)
84005	cemetery 1 (Nunnan)	×1.03	×132.2	XY	H1ap1	I1a1b3 (I-Z74*)
84035	cemetery 1 (Nunnan)	×0.2	×149.6	XX	H2a3a	–
nuf002	cemetery 1 (Nunnan)	×0.16	×44.1	XY	T1a1j	ND
kls001	cemetery 2 (Kålsängen)	×0.13	×11.8	XY	H1b1	R1* (R-M173*)
kal006	cemetery 3 (Kållandet)	×1.2	×87	XX	V7a	–
kal009	cemetery 3 (Kållandet)	×0.19	×124.4	XX	T2f1	–
2072	cemetery 4 (Bensinst.)	×0.01	×1.5	XY	U	ND
bns023	cemetery 4 (Bensinst.)	×0.02	×3.7	XX	H4a1a3a	–
gtm021	cemetery 4 (Götes Mack)	×0.43	×34.1	XX	H5	–
gtm127	cemetery 4 (Götes Mack)	×0.06	×11.1	XX	H1a3a	–
97002	mass grave (St. Lars)	×0.12	×27.7	XY	J2a1a (0.6)	R1b (R-312*)
97026	mass grave (St. Lars)	×0.08	×87.6	XY	U5a2a1	ND
97029	mass grave (St. Lars)	×0.07	×34.2	XY	J1c2	ND
stg020	church 1 (St. Gertrud)	×0.18	×59.4	XX	T2	–
stg021	church 1 (St. Gertrud)	×3.4	×136	XX	J1d1b1	–
stg026	church 1 (St. Gertrud)	×0.61	×367.2	XX	J1c2k	–
grt035	church 1 (St. Gertrud)	×3.2	×279	XY	H	G2a2 (G-L1259*)
grt036	church 1 (St. Gertrud)	×2.2	×247.8	XY	H13a1a5	I2a2/2b (I-M436*)
urm045	church 1 (Urmakaren)	×0.09	×74.7	XY	H1a8	ND
urm160	church 1 (Urmakaren)	×1.3	×299	XY	H1q	R1b1a2a1a1 (R-L11*)
urm161	church 1 (Urmakaren)	×0.08	×19.6	XY	T1 (0.4)	A2′3′4
urm035	church 1 (Urmakaren)	×0.26	×240.3	XY	H2a1c	BCDEF

Mol., molecular; ND, not determined. See also Tables S1 and S3, Figures S2–S4, and Data S1.

As mentioned, the two patterns of genetic variation and variation in strontium isotope ratios depict two different strata: time depth and contemporaneous geographical coverage. For the 16 individuals with both isotope and genetic data available, we combined this complementary information to study individual migration patterns.

Eight of the 16 individuals were confirmed as non-locals with strontium values falling beyond the local range of bioavailable strontium: 0.717–0.732 established for Sigtuna in the present study and also beyond 0.723–0.733 baseline established for the whole of Lake Mälaren region [12]. The correlation between strontium values and local genetic variation predicted using shared drift with modern Norwegians (measured by outgroup f_3 statistics) as a proxy is presented in Figure 3. Out of eight carriers of non-local strontium signatures, four were also genetic outliers (cemetery 2: kls001; cemetery 3: kal006 and kal009; church 1: stg020), suggesting migration from a genetically distinct region (first-generation long-distance migrants). Further four carriers of non-local strontium signatures (cemetery 1: 84001; cemetery 4: gtm127 and 2072; church 1: stg021) fell within or close to the genetic variation of modern Norwegians (Figures 2A and S3C). These curious cases may be considered regional immigrants who came to Sigtuna from other parts of Scandinavia (first-generation short-distance migrants) (Figure 3). Finally, six individuals presented strontium values and genetic variation that agreed with a local origin, in or close to Sigtuna (cemetery 1: 84005 and 84035; cemetery 4: gtm021; mass grave: 97029; church 1: urm160 and stg026). However, two individuals with local strontium signatures (cemetery 1: nuf002; church 1: urm035) fell close to Norwegian and Ukrainian gene pools. Though speculative, it is possible that the two individuals represent second generation of immigrants.

Thus, by combining the strontium values with the genomic data we could identify three groups: the locals (strontium values between 0.717 and 0.732 and falling within genetic variation of modern Norwegians), regional immigrants (strontium values beyond 0.717–0.732 and falling within genetic variation of modern Norwegians), and long-distance immigrants (strontium values beyond 0.717–0.732 and falling outside genetic variation of modern Norwegians), as well as two likely second-generation immigrants (strontium values within 0.717–0.732 and falling on the fringes of genetic variation of modern Norwegians).

Genetic Similarities and Differences between and within Sites

In order to investigate levels of individual variation in Sigtuna we computed outgroup f_3 values between ancient individuals (Figure S4A) and average pairwise distance between pseudo-haploid individuals as a measurement of genetic diversity [31] between and within different groups in Sigtuna, other published Iron and Bronze Age datasets [36, 38], and modern-day populations (Figure 2B; Data S1, sheet 3). To avoid noisy estimates of diversity, we restricted this analysis to ancient samples with 0.8× sequencing coverage or more, which gives an estimate

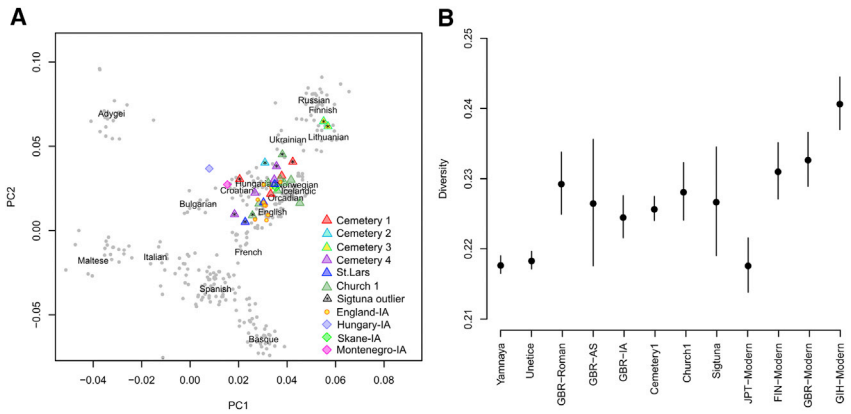

Figure 2. Genetic Variation of Ancient Individuals from Sigtuna

(A) A PCA analyses of Sigtuna individuals together with 13 European Iron Age (IA) samples collected from the literature [34, 36, 38]. The names indicated in the plot represent modern-day populations. See also Figure S3C.

(B) Average pairwise genetic diversity measured in complete Sigtuna, St. Gertrud (church 1) and cemetery 1 (the Nunnan block) compared to both ancient and modern populations ranked by time period (Yamnaya, Unetice, and GBR-Roman, Roman Age individuals from Great Britain; GBR-AS, Anglo-Saxon individuals from Great Britain; GBR-IA, Iron Age individuals from Great Britain; JPT-Modern, present-day Japanese from Tokyo; FIN-Modern, present-day Finnish; GBR-Modern, present-day British; GIH-Modern, present-day Gujarati Indian from Houston, Texas). Error bars show ±2 SEs. See also Table S3, Figures S2–S4, and Data S1.

for Sigtuna as a whole plus estimates for two sites, church 1 and cemetery 1. In Sigtuna, the genetic diversity in the late Viking Age was greater than the genetic diversity in late Neolithic and Bronze Age cultures (Unetice and Yamnaya as examples) and modern East Asians; it was on par with Roman soldiers in England but lower than in modern-day European groups (GBR and FIN; Figure 2B). Within the town, the group excavated at church 1 has somewhat greater diversity than that at cemetery 1. Interestingly, the diversity at church 1 is nearly as high as that observed in Roman soldiers in England, which is remarkable, since the latter was considered to be an exceptionally heterogeneous group in contemporary Europe [39]. To investigate genetic structuring among burial locations (excluding "cemetery 2," represented by one individual only), we tested genetic clustering based on pairwise outgroup f_3 values across all ancient individuals. We found significant variation in within-group outgroup f_3 values (i.e., some burial location groups, such as church 1, showing less homogeneity than others; Kruskal-Wallis, test p = 0.0021). Testing overall differentiation, the mean within-group outgroup f_3 among all burial location groups (median = 0.1562) was higher than would be expected by chance (median = 0.1541, estimated by 100,000 permutations

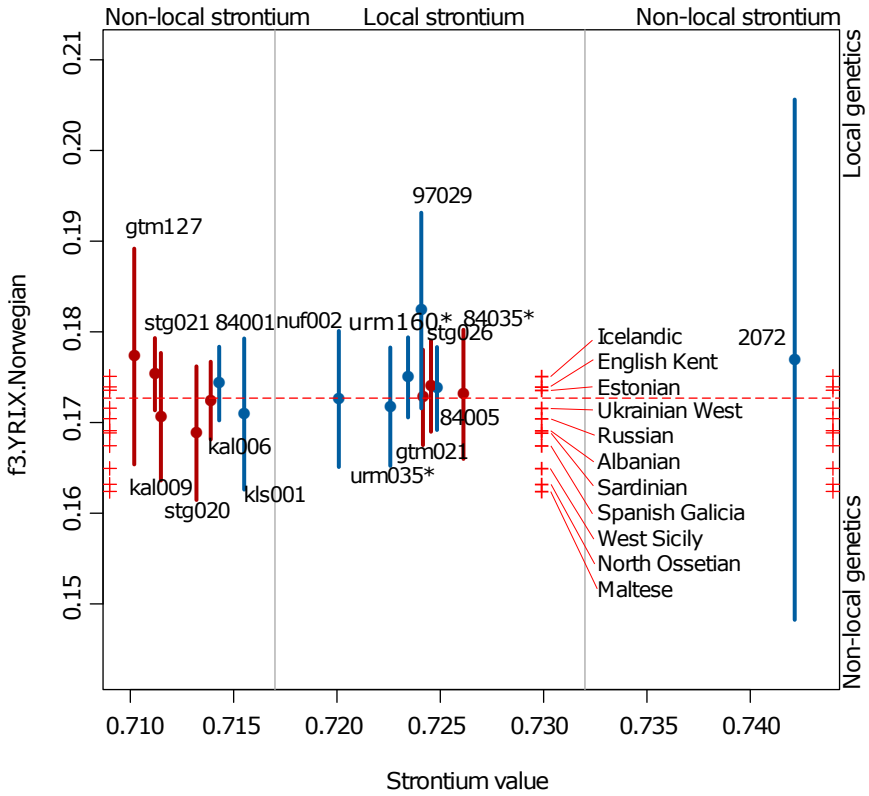

Figure 3. Migration Patterns in Sigtuna Illustrated by a Scatterplot of Individual f_3 Values and Strontium Isotope Values

The plot visualizes of 16 males (blue) and females (red) from Sigtuna with their respective strontium values. All strontium values were measured in the first molar (M1). Individuals marked with asterisk had strontium values measured in the second molar (M2). The vertical gray lines denote local strontium range, thus suggesting local and non-local origin of individuals. The genetic distance is visualized as the amount of genetic drift shared with modern Norwegians. The vertical error bars show ±2 SEs. The f_3 values for the Norwegians and respective European populations (listed in gray) are marked by red crosses at the edges of the plot and are meant to visualize the approximated expected geographical distances as measured by sharing of the genetic signal. See also Tables S1 and S2, Figures S1–S4, and Data S1.

of group identities, p = 0.045), but this slight detected differentiation could not be traced to any two particular groups (Figures S4D and S4E).

Social Structures and Mobility

Different sex-related mobility patterns for Sigtuna inhabitants have been suggested based on material culture, especially ceramics. Building on design and clay analyses, some female potters in Sigtuna are thought to have grown up in Novgorod in Rus' [40]. Moreover, historical sources mention female mobility in connection to marriage, especially among the elite from Rus' and West Slavonic regions [41, 42]. Male mobility is also known from historical sources, often in connection to clergymen moving to the town [43].

Interestingly, we found a number of individuals from Sigtuna to be genetically similar to the modern-day human variation of eastern Europeans, and most harbor close genetic affinities to Lithuanians (Figure 2A). The strontium isotope ratios in 28 adult individuals with assigned biological sex and strontium values obtained from teeth (23 M1 and five M2) show that 70% of the females and 44% of the males from Sigtuna were non-locals (STAR Methods). The difference in migrant ratios between females and male mobility patterns was not statistically significant (Fisher's exact test, p = 0.254 for 28 individuals and p = 0.376 for 16 individuals). Hence, no evidence of a sex-specific mobility pattern was found.

Our results show that the population of Sigtuna was heterogeneous already during the first 200 years of the town's existence, and the observed heterogeneity is expressed in different ways. High levels of genetic variation are accompanied by large variation in strontium isotope ratios, but the two markers do not mirror one another. The observed patterns are best explained by a scenario in which both males and females were mobile regionally but also migrated over larger distances to a similarly high degree. The long-distance migrants probably moved to Sigtuna from other centers in connection to their profession or goals. They most likely represent the whole network of the Viking world. We do not find a specific Scandinavian "Viking" population distinct from the rest of Europe; rather, the population was integrated in the northern European gene pool at the time.

The relatively high genetic and strontium diversity explains the lack of significant genetic sub-structuring between the burial grounds. Previously identified diet-based sub-structuring between burial sites in Sigtuna revealed that individuals from cemetery 1 exhibited significantly lower nitrogen values than those interred in church 1, indicating that the latter group had a diet including more animal protein [44]. As these social groups are not mirrored by our genetic or strontium data, this suggests that the inclusion in them was not based on kinship. Therefore, it appears as if socio-cultural factors, not biological bonds, governed where people were interred (i.e., the choice of cemetery).

The genomic and isotope data from Sigtuna paint a picture of a town composed of inhabitants from a much larger region than the town itself and the local area surrounding it. It represents a single node within a network of similar urban hubs located in various parts of northern Europe at the time. If late Viking Age Sigtuna is representative for those towns, their inhabitants did not consist of distinct homogeneous sub-populations, but should rather be viewed as a cosmopolitan group.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DEATILS
  - Archaeological samples and Sigtuna
  - Signs of long distance contacts in the archaeological record/non-local objects
- METHOD DETAILS
  - Methods for osteological sex and age assessments
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  - DNA Extraction and sequencing
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  - Biological sex assignment and uniparental genetic markers
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Population analyses
  - Functional SNPs
- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, three tables, and one data file and can be found with this article online at https://doi.org/10.1016/j.cub.2018.06.053.

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AUTHOR CONTRIBUTIONS

M.K., A.K., and A.G. conceived and designed the study; M.K. and V.S. performed experiments; M.K., T.G., A.O., M.S., and R.Y. processed and analyzed the genetic data; J.S., A.K., J.E., and C.K. processed and analyzed the isotopic data; M.K., T.G., J.S., V.S., M.S., and G.M.K. prepared the figures; A.K., C.H.-J., T.Z., and J.S. contributed samples and/or provided input about archaeological and anthropological context; M.S., M.J., J.S., and A.G. coordinated the study; and M.K., A.K., J.S., and A.G. wrote the manuscript with input from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
84001	Sigtuna Museum	84001
84005	Sigtuna Museum	84005
84035	Sigtuna Museum	84035
nuf002	Sigtuna Museum	84002
kls001	Sigtuna Museum	05002
kal006	Sigtuna Museum	7006
kal009	Sigtuna Museum	7009
2072	Sigtuna Museum	8072
bns023	Sigtuna Museum	08023
gtm021	Sigtuna Museum	14021
gtm127	Sigtuna Museum	14027
97002	Sigtuna Museum	97002
97026	Sigtuna Museum	97026
97029	Sigtuna Museum	97029
stg020	Sigtuna Museum	83020
stg021	Sigtuna Museum	83021
stg026	Sigtuna Museum	83026
grt035	Sigtuna Museum	95035
grt036	Sigtuna Museum	95036
urm045	Sigtuna Museum	90045
urm160	Sigtuna Museum	90160
urm161	Sigtuna Museum	90161
urm035	Sigtuna Museum	90035
Chemicals, Peptides, and Recombinant Proteins		
Proteinase K	VWR Sweden	Cat#1.24568.0100
EDTA buffer solution pH 8.0 (0.5 mol/L) for biotechnology, sterile	VWR Sweden	Cat#E522-100ML
Critical Commercial Assays		
MinElute PCR Purification Kit	QIAGEN	Cat#28006
High Sensitivity DNA Kit (Bioanalyzer 2100)	Agilent Technologies	Cat#5067-4626
Deposited Data		
84001 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540883
84005 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540884
84035 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540885
nuf002 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540902
kls001 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540900
kal006 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540899
kal009 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540891
2072 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540882
bns023 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540901
gtm021 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540903
gtm127 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540904
97002 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540886
97026 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540887

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
97029 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540888
stg020 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540892
stg021 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540893
stg026 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540894
grt035 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540889
grt036 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540890
urm045 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540896
urm160 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540897
urm161 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540898
urm035 hg19 BAM file	European Nucleotide Archive (ENA)	ENA: ERS2540895
Oligonucleotides		
IS1_adapter.P5: 5′-A*C*A*C*TCTTTCCCTACACGAC GCTCTTCCG*A*T*C*T-3′ (* indicates a PTO bond)	[45]	Biomers
IS2_adapter.P7: 5′-G*T*G*A*CTGGAGTTCAGACGT GTGCTCTTCCG*A*T*C*T-3′ (* indicates a PTO bond)	[45]	Biomers
IS3_adapter.P5+P7: 50-A*G*A*T*CGGAA*G*A* G*C-30 (* indicates a PTO bond)	[45]	Biomers
IS4: (5′-AATGATACGGCGACCACCGAGATCTAC ACTCTTTCCCTACACGACGCTCTT 3′)	[45]	Biomers
P7 indexing: (5′-CAAGCAGAAGACGGCATACGAGAT xxxxxxxGTGACTGGAGTTCAGACGTGT 3′) where x is one of 228 different 7 bp indexes	[45]	Biomers
Software and Algorithms		
MergeReadsFastq_cc.py	[46]	https://bioinf.eva.mpg.de/fastqProcessing/
FilterUniqSAMCons_cc.py	[46]	https://bioinf.eva.mpg.de/fastqProcessing/
Burrows-Wheeler Aligner BWA aln version 0.7.8	[47]	http://bio-bwa.sourceforge.net/
SAMtools-0.1.19	[48]	https://sourceforge.net/projects/samtools/files/samtools/0.1.19/
Haplofind	[49]	https://haplofind.unibo.it/new/
PhyloTree Y (30th Nov 2014)	[50]	http://www.phylotree.org/
Network	[51]	http://www.fluxus-engineering.com
PMDtools	[52]	http://code.google.com/p/pmdtools/
HIrisPlex	[53–55]	http://hirisplex.erasmusmc.nl/
Other		
ATP	Fermentas/Thermo Scientific	Cat#R0441
T4 Polynucleotide Kinase	Thermo Scientific	Cat#EK0032
T4 DNA Polymerase	Fermentas/Thermo Scientific	Cat#EP0062
Bst polymerase (supplied with 10X ThermoPol reaction buffer)	NEB/BioNordika	Cat#M0275S
AmplitTaq Gold	Invitrogen/life technologies	Cat#4311816
ATP	Fermentas/Thermo Scientific	Cat#R0441
10X Tango Buffer	Fermentas/Thermo Scientific	Cat#BY5
Maxima SYBR Green/ROX qPCR Master Mix	Fermentas/Thermo Scientific	Cat#K0221
T4 DNA Ligase	Fermentas/Thermo Scientific	Cat#EL0011
Min Elute PCR Purification Kit	QIAGEN	Cat#28006
PEG-4000	Sigma	Cat#1546569
USER enzyme	NEB/BioNordika	Cat#M5505L
AccuPrime Pfx DNA Polymerase (2.5U/μL)	Fermentas/Thermo Scientific	Cat#12344024
Agencourt AMPure XP beads (60 mL)	Beckman Coulter	Cat#A63881

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Maja Krzewińska (maja.krzewinska@arklab.su.se).

EXPERIMENTAL MODEL AND SUBJECT DEATILS

Archaeological samples and Sigtuna

Bone and teeth from 23 individuals from six different burial sites were sampled and obtained bone powders were used for DNA extraction. The individuals were recovered in archaeological excavations led over many years from 1983 to 2008 [23, 25]. Osteological and archaeological details are listed in Table S1.

Sigtuna is located in the Mälaren Valley in east-central Sweden approximately 50 km from Stockholm (59°36'N, 17°42'E). The town was founded in the second half of the 10th century CE and is the oldest, still existing town from medieval Sweden. Sigtuna witnessed many archaeological excavations during the latest century and more than 800 graves have been unearthed, resulting in a number of studies describing in depth living conditions and dietary practices. Efforts to classify different burials deposited over centuries and associated with the church topography led to the differentiation of three burial phases in Sigtuna [22]. The phases have been roughly chronologically defined; phase 1 (ca 970 CE-11th century), phase 2 (ca 12th-13th century) and phase 3 (ca 14th century –1530 CE). Most of the individuals were buried in graveyards associated with churches (churchyards) of which there were between eight or ten in the town. Some burials were located in graveyards without churches, here simply named 'cemeteries' (e.g., the Nunnan block), though with a Christian expression. Sigtuna is considered Christian from the foundation, and only a single cremation burial has been found.

Excavations in Sigtuna have revealed a large number of parallel town plots with several houses, showing that the town was most likely planned and organized already from the beginning [56]. The absence of a major harbor constructions, the few finds of an agrarian character, and lack of craft activities in the early town have been taken as an indication that Sigtuna foremost functioned as an administrative center for the royal Christian power. Several cemeteries and churchyards are distributed in a semicircle around the town area; these have been excavated at different times since the 1920s [56].

In the current study 23 and 36 skeletons from six sites, all from the first burial phase (late 10th to 12th the century), were sampled for DNA and strontium isotope analyses respectively (Table S1). Four sites were cemeteries without a church building, while one site was a churchyard and one was a mass grave: Cemetery 1 (The Nunnan block), Cemetery 2 (The Kålsängen block), Cemetery 3 (The Kållandet block), Cemetery 4 (Bensinstationen and Götes mack), Church 1 (St Gertrud and Urmakaren), and Mass grave (St. Lars Church) [21–25].

Cemetery 1 (The Nunnan block)

The cemetery was situated at the western outskirts of the town. The burials clustered into three groups consisting of 49, 41 and 29 graves, altogether 119 individuals, which could represent different populations, family groups or extended households. Although under flat ground the graves were sparsely distributed with almost no intersections indicating that they originally had been marked by a cross or the like. Many burials showed signs of wooden coffins, some contained charcoal and about 20% revealed the remains of clothing or personal items. The objects were dress items, such as beads, knives, strike-a-lights and brooches; among them was also a pendant from the Ladoga region and four English and German/Frisian coins, and an additional found during conservation [57]. Five individuals were placed in a crouched position as if buried according to Slavonic tradition. The burial ground was likely in use from 970/80 CE to 1060/80 CE.

Cemetery 2 (The Kålsängen block)

Excavations have revealed two inhumation burials at the site. At least one of the graves had a wooden coffin, both graves contained charcoal. The deceased were a man who, although he died young, showed signs of having been exposed to physical stress, and a child of c. two years old, small for its age perhaps due to bad health [25].

Cemetery 3 (The Kållandet block)

Of the eight recovered individuals, six were adults (four women and two unsexed individuals) and two were children, six and nine months old [23]. Wooden coffins were found in three of the burials, two of the graves contained charcoal, and the graves lay dispersed.

Cemetery 4 (Bensinstationen and Götes mack)

The cemeteries known as "Götes mack" and "Bensinstationen" are part of the same larger Christian cemetery. No burials had remains of clothing or personal items and 84% of the individuals were buried in wooden coffins [24]. One of the coffins was a reused side of a boat. The 14C-datings of two burials gave the date 890–1030 CE (Ua-37645: 1065BP ± 35; Ua-37646: 1060BP ± 35). The cemetery held 52 individuals including men, women and children [21, 24]. The burials as observed in other early cemeteries seem to have been oriented toward the road that led through the town [24]. Moreover, the cemetery was spaced according to sex with females buried mostly in the northern part, and males in the southern part [21].

Church 1 (St Gertrud and the Urmakaren block) (previously known as Bishop's Church)

The church (and associated churchyard) is considered by many scholars to be the oldest in Sigtuna [19]. It was situated in the midst of the town but placed higher than the other town plots. This site had probably earlier housed the first royal manor [56]. The churchyard

has an irregular unusual shape [22]. The graves belonging to Church 1 are of two distinct types: one group is oriented east-west according to the apsidal stone church and have lime mortar in the grave fillings, whereas the other group does not have any lime mortar in the fillings and is oriented northwest-southeast [43].

The absence of lime mortar in the latter group suggests the existence of an earlier wooden church on the site. The fact that there are burials under the churchyard wall and that several burials were damaged during the construction of the wall suggests that the earliest cemetery had a different layout. The dating of the stone church has been debated, but its erection probably started around 1025-50 CE [43].

In 1993, near the southern church wall, a buried bishop furnished with a crozier, was discovered. The burial probably dates to the early 12th century. The remains of a baptismal font of sandstone of an exceptional quality from the same time period have been found in the churchyard. These factors imply the high social status of the church and that it was the main church of the archdiocese. The church was demolished by the end of the 13th century at the latest [43].

The Mass grave (St. Lars Church)

A mass grave older than the stone church of St. Lars was found in the south-east end of the churchyard [22]. It seems to have been respected throughout time and was not superimposed by later burials. In the mass grave at least 19 individuals were buried, including men, women and children and the majority showed signs of sharp force trauma. The grave is dated to around 990 CE. Sustained injuries indicate that the victims were unarmed and not able to defend themselves [22]. The assault seems to have taken place at the very beginning of the town's existence.

Signs of long distance contacts in the archaeological record/non-local objects

Since the beginning of the 20th century, Sigtuna has been subject to several archaeological investigations. The numerous excavations have produced an extensive find material indicating the diverse character of the site. The various elements of the material culture put together confirm that Sigtuna already during its initial phase (i.e., 10th – 12th century) had long-term long distance contacts of varying character. Local and regional types occur together with objects with a distinct imported character. The latter group of objects derives from several different contexts and reflects different levels and groups of Sigtuna's early medieval society. They comprise prestigious goods and religious paraphernalia as well as everyday objects. Their provenance and life histories vary and where some of them reached Sigtuna as imports, others may have been produced in the town by foreign artisans and craftsmen or by local craftsmen who were inspired by international styles, forms and decorations. The provenances of the imported objects cover the area from the Black Sea region and Byzantium in the East through most of Central Europe, to Anglo Saxon England in the West. Table S2 presents a selection of objects from Sigtuna's earliest phase representing different aspects and levels of society and their general region of provenance [56].

METHOD DETAILS

Methods for osteological sex and age assessments

International standard methods for morphologic indicators were applied for sex and age estimations [58]. To estimate the age of young individuals dental development were recorded according to Moorrees et al. [59], Ubelaker [60] and Smith [61]. The degree of epiphyseal ossification and union were classified according to Scheuer and Black [62]. For adults morphological changes of the auricular surface and pubic symphysis were evaluated [63–65]. Additionally, cranial suture closure [58, 64] and dental attrition were recorded [66]. The sex assessments were based on morphological features of the crania and on the innominate bones [58, 67–69].

Strontium isotope analyses

Introduction

Sigtuna seems to have attracted people from different areas to visit and settle in the town. The archaeological record witnessed faraway contacts and the town was the most important urban hub in this part of Scandinavia. Thus, strontium isotope analyses in combination with the genetic data would aid interpretations and help understand the demographic history of the inhabitants in early Sigtuna in the 10th to 12th century CE. The aim was to address questions concerning human mobility in Sigtuna by analysis of strontium isotope ratios ($^{87}Sr/^{86}Sr$) in human tooth enamel in order to be able to identify individuals with a local or non-local geographic origin. The potential of such studies, highlighting varying mobility patterns, in various prehistoric and historical contexts has been confirmed before [20, 70–73]. In Sweden, strontium isotope analyses have been performed mainly in the southern parts, often on human remains from older time periods [74–76]. A limited number of studies deal specifically with Sweden [77]. The analytic methods involved for strontium isotope analyses have been described in several publications [73, 78–80]. Analysis of stable isotopes (Sulfur) of individuals buried at the Viking proto-town at Birka showed that some individuals were of non-local origin as judged from $\delta^{34}S$ values [10].

Strontium is incorporated into the skeletal tissue through the food chain where it replaces calcium in the bone or tooth enamel matrix. As tooth enamel is not remodeled the $^{87}Sr/^{86}Sr$ ratio remains unchanged through life and the ratio is commonly not extensively affected by diagenetic alteration. ^{87}Sr is produced by the radioactive decay of rubidium (^{87}Rb) over time [81] and the isotope ratios are characteristic of the local bedrock and soil conditions. Thus, the strontium isotope ratio found in bone and teeth corresponds to the local bedrock [73, 82]. It is important to focus attention on the biologically available strontium as local isotope ratios may differ

significantly from those of a whole rock analysis of a sample from the very same location. Hence, differences in the ratios of different teeth would indicate a change in residence, i.e., mobility and residential change during childhood or adulthood or multiple movements during childhood. Differences between $^{87}Sr/^{86}Sr$ ratios of enamel in general and the bio-available strontium at a given locality indicate a residential change between childhood and adulthood. Analyses of enamel from different teeth may resolve different events of residential relocations during childhood.

In order to relate the strontium isotope values in the human teeth to the regions the local bioavailable strontium isotope ($^{87}Sr/^{86}Sr$) baseline needs to be established as a reference for comparisons. The bedrock in large parts of Eastern Middle Sweden belongs to the Baltic Shield and comprises (old) Precambrian crystalline (granitic) rocks but also younger metamorphic rocks, such as sandstone and quartzite [83]. Older rocks usually have a higher $^{87}Sr/^{86}Sr$ ratio while younger rocks exhibit lower ratios [82]. Background data from Eastern Middle Sweden is available from lake and river waters and they show that the area may be expected to exhibit rather high variation in strontium isotopic values. River-water draining from these Precambrian rocks exhibits $^{87}Sr/^{86}Sr$ values between 0.721-0.745 [84, 85]. A recent study reported estimated base-line $^{87}Sr/^{86}Sr$ values for an area with similar bedrock type around 150 km North of Sigtuna, in Sala between 0.720 and 0.740 [86].

Earlier published strontium isotopic data of local baselines in other parts of Sweden are available in e.g., Sjögren et al. [75] and Eriksson et al. [74]. Corresponding data on human tooth enamel from the Iron Age Viking town of Birka and other parts of Eastern Middle Sweden are also available [11, 12, 73, 77]. In a sample of 42 individuals from the Viking Age town of Birka, located 30 km south from Sigtuna exhibited the strontium isotopic ratios varied between c. 0.7103 and c. 0.7343 [12]. The strontium rations in tooth enamel of an additional five individuals from Birka varied between 0.71220 and 0.726 [11]. The analyzed individuals include a number of non-local individuals as the estimated local baseline of bioavailable strontium in the Mälar region has been estimated to 0.723-0.733 [12]. However, the above given ranges cannot be directly used as comparison for Sigtuna but still give a rough indication of the variation in the isotopic ratios in human teeth from roughly the same time period and region as well as the local baseline. We here provide additional data of human bones in order to further clarify the isotopic baseline ratio for Eastern Middle Sweden and especially suitable to use for Sigtuna.

The analyses explore mobility during childhood and adolescence, i.e., during the timing of the formation of the tooth crows. The permanent molars are mineralized broadly from c. 0.5 months in utero to c. 3 years for the first molar (M1), 2-3 years to c. 8 years for the second molar (M2) and finally 7-16 years for the third molar (M3) [87, 88].

Material

Samples of teeth of 31 individuals from different cemeteries in Sigtuna were submitted for strontium isotope analyses (Table S1). From 23 of these genetic information is available. Due to fragmentation and (bad) preservation all three molar teeth were available for analysis in 11 individuals (males = 9, females = 2), while one or two molars were available for 17 individuals (males = 9, females = 8). Additionally three sub-adults (whereof one is a girl) with one or two molars are included. Bone samples were taken from five additional individuals, thus, the total sample analyzed for strontium was 36 individuals.

Samples were analyzed by the Curt-Engelhorn-Centre for Archaeometry gGmbH, Mannheim (CEZA; Dr. Corina Knipper) and by NERC Isotope Geosciences Laboratory British Geological Survey, Keyworth, Nottingham, NG12 5GG, UK (Prof. Jane Evans). The analyses targeted the ^{87}Sr and ^{86}Sr isotope ratios in the enamel of molar teeth but also bone samples. For a few individuals, the first molar was replaced with an incisor or a canine that form roughly during the same age (Table S1). As bone values are often altered by diagenetic processes, their isotopic ratios do not always represent biogenic values from the last years of live of an individual. They may, however, mimic the local signature of the place of deposition and therefore represent valuable baseline data. We thus analyzed human bone samples from 17 burials and enamel of two pig teeth in order to evaluate the local biologically available $^{87}Sr/^{86}Sr$ isotope ratios. Using animal teeth in a setting such as Sigtuna may be complicated as most of the animals probably originated from the nearby area and not from the town area itself. The bone samples were chosen from several cemeteries in order to capture many different contexts and, thus, possibly a variation as wide as possible in the strontium isotope ratios.

Laboratory methods

Sample preparation at CEZA followed the methods described in Knipper et al. [71, 89] and comprised the following steps: The teeth to be sampled were selected from complete or fragmented mandibles or maxillae that had been sent to CEZA. Enamel fragments were cut from the crowns using a diamond-coated cutting disc attached to a dental drill and all surfaces and remaining dentine were removed using diamond-coated milling bits. The enamel chips were ground in an agate mortar, while bone samples were directly milled as powder. The samples were pre-treated with 0.1 M acetic acid buffered with lithium acetate (pH ca. 4.5) and washed three times with Milli-Q water. During each of these steps the samples were placed into an ultrasonic bath for 10 min. Samples were afterward dried overnight (50°C) and reduced to ashes to remove remaining organic components (3 hr at 850°C). All subsequent steps were carried out under clean lab conditions. The samples were dissolved in nitric acid (3N HNO_3) and the strontium separated using Sr-Spec ion exchange resin. Strontium concentrations were determined using a Quadrupol ICP mass spectrometer, the solutions diluted, and the isotope ratios determined using a High Resolution-Multi Collector-Inductively Coupled Plasma-Mass Spectrometer (HR-MC-ICP-MS; Neptune). The raw data were corrected according to the exponential mass fractionation law to $^{88}Sr/^{86}Sr = 8.375209$. Blank values were less than 10 pg strontium during the whole clean lab procedure, including digestion, strontium separation and measurement. NBS-987 run the samples produced an average of 0.71024 ± 0.00004 (2 SD; n = 41) and the Eimer and Amend standard (AMES) produced 0.70803 ± 0.00004 (2 SD).

At NERC the enamel surface of the tooth was abraded to a depth of >100 microns using a tungsten carbide dental burr and the removed material discarded. Thin pieces of enamel were then cut from the tooth using a flexible diamond edged rotary dental

saw. All surfaces were mechanically cleaned with a tungsten carbide burr to remove adhering dentine. The resulting samples were transferred to a clean (class 100, laminar flow) working area for further preparation. In a clean laboratory, the sample was cleaned ultrasonically in high purity water, rinsed, then placed on a hot plate at c 60°C for a further clean in de-ionized water and then dried and weighed into pre-cleaned Teflon beakers. The samples were mixed with ^{84}Sr tracer solution and dissolved in Teflon distilled 8M HNO3. Strontium was collected using Dowex resin columns. Strontium was loaded onto a single Re Filament with TaF following the method of Birck [90], and the isotope composition and concentrations were determined by Thermal Ionization Mass spectroscopy (TIMS) using a Thermo Triton multi-collector mass spectrometer. The international standard for $^{87}Sr/^{86}Sr$, NBS987, gave a value of 0.710251 ± 0.000005 (n = 19, 2*) during the analysis of these samples. Blank values were in the region of 70pg.

Results

The $^{87}Sr/^{86}Sr$ ratios of the human bones from Sigtuna varied between 0.7199 and 0.7296 with one outlier at 0.7354 (burial 97026), while enamel from two pig teeth exhibited values of 0.7185 and 0.7276. Possibly, the pre-treatment of the bones in burial 97026 was effective and this bone in fact may have retained a biogenic value of a non-local individual. The mean value of these samples is 0.7245 (SD 0.0039), suggesting a range between 0.7167-0.7323 (Mean ± 2SD) for the regionally biologically available strontium isotope ratios (i.e., local baseline). This range is somewhat wider than that estimated in Price et al., 0.723-0.733 (2018) and especially the lower end value is different in Sigtuna. The lower end value in Sigtuna is here conservatively estimated to 0.717 (based on the pig tooth ratio of 0.718 and the mean-2SD of 0.7167 for the bone values) and the upper at < 0.732 (mean+2SD for human bone values). More analyses are still needed to evaluate the full variation of the biologically available $^{87}Sr/^{86}Sr$ values in the Mälar region but also the isotopic ratios in different regions of Eastern Middle Sweden. Still, the data at hand allow for the identification of values in human tooth enamel that may be considered non-local to the region.

Of the 36 individuals analyzed 26 had preserved first molars. Their $^{87}Sr/^{86}Sr$ ratios vary between 0.70852 and 0.74215, i.e., a variation larger than for the bone values (Table S1 and Figures S1B and S1C). When the values for the first molars are considered, 15 of the individuals presented strontium isotope ratios outside of the limit of the estimated local base-line. An additional five individuals that lack the first molars but instead have preserved second molars may be included in the evaluation. Two of these individuals exhibit a non-local isotope ratio. However, the two individuals (84012 and 97016) exhibit isotope ratios that fall close to the lower end of the local baseline and, in fact, the value for the third molar of 84012 falls within the range. A possible further calibration of the local baseline will probably not change the general pattern of the high frequency of people who died in Sigtuna, but were born elsewhere.

The limited intra-individual variation in $^{87}Sr/^{86}Sr$ ratio for different molars between 0.00001 and 0.01151 (mean 0.00289) indicates that most of the considered individuals were residents and grew up in the same geographical area between c. 0-16 years of age, (Figure S1C). Most of the individuals of a non-local origin moved to Sigtuna after the age of the formation of the third molar (c. 16 years), i.e., apparently as adults. There are at least three exceptions; one male (8072) probably moved to Sigtuna between the age of the formation of the second and third molar. Further, two individuals obviously moved to Sigtuna as children; individual 14011 died already as a young teenager and individual 97025 died around 8 years of age from a violent cause. Thus, the isotopic data at hand indicate that most often the individuals that moved to the town were adults. However, more data of those that died as children is needed to better evaluate the age of migration/mobility.

Considering the 31 individuals with available data on M1 or M2 (when M1 was absent), the strontium isotope ratios indicate that up to 55% (17 of 31) of the population in Sigtuna may have been of non-local origin. The sample size is small, but there is a marked difference between the adult females and males in the proportion of non-locals versus locals; 70% (7 of 10) exhibit non-local isotopic ratios among the females and 44% (8 of 18) among the males (Table S1 and Figures S1D and S1E). Thus, the results of the strontium isotope analyses show that a considerable part of the inhabitants buried in Sigtuna traced their origin outside of the town. In fact, the town did not attract inhabitants from the nearby region only, but probably also from rather far away. The isotopic ratios of most of the non-locals fall below the local baseline – indicating an origin with rather different bedrock than Eastern Middle Sweden. Many values fall close to that observed, for instance on Gotland [77] but also some parts of southern Sweden [75]. However, it is not possible to pinpoint an exact area of origin. Likely the main areas may be sought to the south and even outside of Scandinavia (compare baseline data in e.g., Price et al. [72, 77]) (Figure S1B). The geographical origin of the two individuals exhibiting the highest ratios might be sought to the North but this remains speculative. It is likely that human mobility followed established routes of communication and both social and geographical networks linked the early urban centers of this time period. The marked variation in geographic origin as indicated by the strontium isotope ratios is in good agreement with the genetic data. The two datasets highlight different aspects of mobility and geographic origin of the inhabitants of Sigtuna.

DNA Extraction and sequencing

DNA extraction and library preparation was undertaken in specialized ancient DNA facilities at the Archaeological Research Laboratory (AFL), Stockholm University. DNA was extracted from bones and teeth using modified extraction method [91] and purified with silica-based spin columns [92]. DNA was extracted from bone or teeth from individuals selected for the analyses (Table S3). One round of DNA extractions was performed for all samples from between 60-130 mg of bone powder. Tooth samples underwent decontamination procedure with 0.5% sodium hypochlorite solution, while the surface of bone fragments was removed mechanically. All osteological material was decontaminated under UV light (6J/cm^2 at 254nm). The DNA was extracted using modified Yang buffer (0.5M EDTA pH 8, 1M Urea, 100 μg/mL proteinase K) and purified with QIAGEN MinElute PCR Purification Kit (QIAGEN) [31, 37, 92–94]. DNA extracts were used for preparation of genomic DNA libraries following Meyer and Kircher [45]. Authors recommendations were followed with the exception of the first step (aiming at fragmentation of large DNA molecules into shorter fragments

appropriate for Illumina HiSeq (<600bp) sequencing) due to the ancient DNA nature of our source material. In short, double-stranded P5 and P7 adapters are ligated to DNA fragments. Generated libraries were inspected on 1% agarose gel (0.5x TBE, stained with GelRed x10000, Biotium Inc.) or by Real Time PCR (BioRad) using IS7 and IS8 amplification primers. Subsequently, the libraries were amplified with AmpliTaq Gold DNA Polymerase (Applied Biosystems) in six separate PCR reactions with different (8-16) number of cycles and using indexing primers containing 7-bp DNA tag sequences. The amplified products were pooled, purified with magnetic beads (Agencourt AMPure XP, Beckman Coulter); quantified using DNA High Sensitivity Kit with Agilent 2100 Bioanalyzer Instrument (Agilent Technologies). The libraries underwent sequencing at the Science for Life Laboratory in Stockholm (administrated by Royal Institute of Technology, KTH). As a rule, the libraries were first screened in pools containing on average 10 individual indexed libraries on Illumina HiSeq 2500 High Output mode v4, with paired-end read runs of up to 125bp in length (PE 2x125bp). Obtained raw screening data was demultiplexed, quality-controlled and delivered to UPPNEX (UPPmax NEXt Generation sequence Cluster & Storage) [95].

Sequence alignment, processing and authentication

All computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Projects b2013203, b2013240 and b2015307. Sequence data was analyzed following Skoglund et al. [31] and Günther et al. [96]. In short, pair-end either 125bp or 150bp sequencing reads were merged and the adapters were trimmed using Martin Kircher's MergeReadsFastQ_cc.py script [46] and the resulting sequences were mapped to the human reference genome using BWA v. 0.7.13 *aln* with non-default parameters settings (-l 16500 -n 0.01 -o 2) [47]. PCR duplicates, sequences shorter than 35bp and those with more than 10% mismatches to the human reference genome were removed resulting in consensus BAM files.

As part of the standard pipeline, all DNA fragments were routinely checked using PMDTools [52] for presence of 3′ and 5′ degradation patterns characteristic of ancient DNA [97, 98], Figure S2. Levels of contamination were estimated based on rare (<5%) mtDNA polymorphic site variation (patters of rare polymorphic sites established on 311 modern reference panel of mitochondrial genomes) allowing for identification DNA mixes indicative of presence of contaminating human DNA [99].

We further evaluated the contamination in male samples based on the fact that they carry only a single copy of the X chromosome. Using a maximum likelihood method described in Rasmussen et al. [100] and implemented in ANGSD [101] (http://www.popgen.dk/angsd/index.php/ANGSD) we estimated the rate of contamination in male samples. We first run ANGSD as ''angsd -i BAMFILE -r X:5000000-154900000 -doCounts 1 -iCounts 1 -minMapQ 30 -minQ 30.'' Using the output of this run (angsdput.icnts.gz), an R script (contamination.R) (https://github.com/ANGSD/angsd/tree/master/R) and reference files provided in ANGSD package (https://github.com/ANGSD/angsd/tree/master/RES) we calculated the rate of contamination as ''Rscript contamination.R mapFile=''chrX.unique.gz'' hapFile=''HapMapChrX.gz'' countFile=''angsdput.icnts.gz'' minDepth=1 maxDepth=100.'' The contamination estimates are listed in Table S3.

Biological sex assignment and uniparental genetic markers

Biological sex

The biological sex of ancient individuals was estimated from genome-wide sequence data by the analyses of the ratio of sequences aligned to sex chromosomes [102]. The method was designed for analyses of shotgun sequencing results. The ratio of sequence alignments to the two sex chromosomes is calculated as $R_Y = n_Y/(n_X + n_Y)$, where n_X is the number of alignments to chromosome X, n_Y is the number of alignments to chromosome Y. The ratio and (R_Y) between n_Y and the sum of all alignments denotes females when $R_Y \leq 0.016$ and males when $R_Y \geq 0.077$ [102]. Finally, a 95% confidence interval (CI) is computed by approximation as $R_Y \pm 1.96 \times R_Y \times (1- R_Y)/(n_X + n_Y)$. All values are presented in Table S3.

Mitochondrial DNA haplogroups

We obtained mtDNA sequence with mean coverage between 1.5- and 367-fold per individual. From this data, we called consensus mitochondrial sequences of each individual using the mpileup and vcfutils.pl (vcf2fq) tools in the SAMtools package with default parameters [48]. We determined the mitochondrial DNA haplogroups of the individuals based on SNPs at informative nucleotide positions of the mitochondrial genome sequences (Data S1, sheet 1). To detect these nucleotide differences and positions, we aligned mtDNA sequence of each individual to the RSRS [103] as reference sequence. Then we identified the polymorphisms and assigned the mtDNA haplogroups of the individuals using HaploFind [49].

We observed haplogroup H, one of the most abundant haplogroup in present-day Europe and in the Near East populations [104, 105], in twelve individuals. Three belonged to subtypes of T2 (T2, T2f1 and T2b4b) and one belonged to T1 (T1a1j). One individual belonged to V7a. Three belonged to subtypes of J (J1c2, J1c2k and J2a1a1a) and haplogroup J1d1b1 which is another subtype of J, was found in one individual. The remaining two individuals belonged to the most common haplogroup in hunter-gatherer populations, U5 (U5a1a1a and U5a2a1).

Y chromosome haplogroup assignment

Y-chormosome haplogroups were assigned based on biallelic SNP markers included in minimal PhyloTree$_Y$ (version 30-Nov-2014) [50]. The nomenclature used includes both branch names as defined in PhyloTree$_Y$ and nomenclature according to International Society of Genetic Genealogy (ISOGG) Y-DNA haplogroup tree 2017 version 12.29. In short, biallelic haplogroup defining SNPs were filtered out from bam files aligned to the human reference genome 19 (hs37d5) using SAMtools v.0.1.19 [48]. Only SNPs of base and mapping qualities of 30 were used. The haplogroup assignment was restricted to derived transversions thus avoiding positions which

could reflect post-mortem damage. However, in cases where restricted genome coverage did not allow for haplogroup assignment we resort to using transition sites to supplement Y haplogroup discussion. Transversion based haplogroup assignment was possible in seven cases, one individual was assigned to major haplogroup using derived transitions, while the remaining six males could not be assigned with confidence to any major Y chromosome haplogroup (Data S1, sheet 2).

We observed haplogroup R1b, in three of the eight male individuals for which the haplogroup assignment was possible. R1b is one of the most common haplogroups in Europe and has been previous pointed out as one of the most common haplogroups in Scandinavia and Northern Europe [28, 29, 106, 107]. Three more individuals were assigned to major Y chromosome haplogroup I*. Two of those assignments could be further narrowed down to haplogroup I1* major I component in Scandinavia, while one male (grt035) was assigned to a rare I2a2 haplogroup, previously identified among Scandinavian hunter-gatherers [37]. The other individual buried in the vicinity (grt036) was a carrier of another widespread European haplogroup G2a2 [108]. Finally, individual 84001 was a probable carrier of N1a1a, a haplogroup associated with East Baltic region (Tables 1 and S3; Data S1, sheet 2).

QUANTIFICATION AND STATISTICAL ANALYSIS

Population analyses

SNP-based population analyses

Obtained results were compared to a wide variety of modern and ancient published genomes. Modern populations used were from the following datasets: the Human Origins dataset [32, 33], and the 1000 Genomes Project (1KGPomn), genotyped at ~2.3 million positions [109]. The ancient reference data was collected from Roman and Iron Age England [36, 38], as well as Iron Age Sweden and Montenegro [34].

To allow for population genetic analyses the ancient samples were merged with the upper- mentioned datasets with the exclusion of transition sites and randomized allele selection at all polymorphic sites. Resulting SNP datasets were used in investigation of genetic affinities among the ancient individuals using Principal Component Analyses (PCA), *D*- and *f3*-statistics and rare allele sharing [36]. All computations were performed using UPPMAX resources (Uppsala Multidisciplinary Centre for Advanced Computational Science) under the following projects: b2013203, b2013240 and b2015307.

Principal Component Analysis

As a means of visualizing genetic affinities of the investigated individuals we have performed the principal component analyses (PCA) including a data-set consisting of 17 modern European populations from Human Origins as a reference population panel [32, 33]. In short, identical analyses were performed for each of the ancient individuals presented here (Figures S3B and S3C) as well as a number of published ancient European individuals dated to the Iron Age, including 10 individuals from England [36], one from Hungary [35] and one from Montenegro [34]. Afterward all ancient individuals were projected onto a single PCA plot using Procrustes transformation [37] (Figure 2A). The analyses were undertaken using *smartpca* module of EIGENSOFT v.6.0.1 [110].

Outgroup f3 and Patterson's D

To obtain information on individual's relatedness to the modern and ancient populations we performed *f3*-outgroup statistics of the form (O; A, B) using qp3Pop v. 204 implemented in ADMIXTools [33]. The outgroup (O), is expected to be equidistant to both the tested sample (A) and any of the other European populations from the reference panel (B). Thus the result of outgroup *f3*-statistics (assuming no gene-flow between the test populations and the outgroup) reflects the amount of genetic affinity shared between test populations, A and B. The analyses were performed with the Human Origins data-set (using between 1186 and 92261 SNPs). The outgroup (O) was the Yoruban population, the tested individual was A, and any of the populations from the reference panel was the test population B. The analyses were performed with up to 704 jackknife blocks. The results are summarized in Figure S3D and are listed in Data S1, sheet 7.

Finally, we tested for deviations from tree-like population history between the analyzed individuals. A statistic for testing tree topologies called *D*-statistics was used as implemented in ADMIXTools [33, 111]. In short, each ancient individual was placed in a treelike topology with Yoruban population as an outgroup and two modern populations on the opposite braches of the test (O, X; A, B). Deviations from tree-like topology can be caused by gene-flow between populations pointing to closer affinity among pairs of tested samples. The standard errors were estimated by performing block jackknife over blocks of the genome [111]. The results are summarized in Data S1, sheet 8.

Conditional nucleotide diversity

In order to get an unbiased estimate of genetic diversity within groups, we used the conditional nucleotide diversity introduced by Skoglund et al. [31]. The low coverage data in ancient DNA research complicates per-individual estimates of heterozygosity, conditional nucleotide diversity avoids this problem by using two pseudo-haploid individuals and a pre-defined set of known polymorphic sites. Per individual and site, one read is randomly drawn as explained above; an estimate of genetic diversity can be obtained by dividing the number of mismatches between the two individuals by the total number of sites with sequencing coverage in both individuals. We merged the ancient individuals with a set of 1,785,224 transversion polymorphisms with a minor allele frequency of at least 10% in the 1000 genomes Yoruban population [109] as described above. Standard errors for conditional nucleotide diversity estimates were calculated using a block-jackknife with a block size of 2000 SNPs.

For Figure 2B, we calculated the average diversities from all pairwise values for a number of prehistoric groups with genome-wide shotgun sequencing data available. Standard errors per group/site were calculated as the square root of the sum of squared standard errors of all pairwise comparisons. The groups/sites and the individuals assigned to them are shown in Data S1, sheet 3, only

individuals with at least 0.8x sequencing coverage were included to reduce noise. The individual 6DRIF-26 has been shown to be a genomic outlier compared to other Roman samples [38], which is why we excluded it from the GBR-Roman group. Modern Eurasian populations from the 1000 genomes project [109] were added for comparison, the values for the modern populations are based on five randomly drawn pseudo-haploidized individuals per population.

Outgroup f_3 heatmap

To display shared drift between pairs of ancient individuals, we calculated pairwise outgroup f_3-statistics: *f3*(YRI; ancient individual1, ancient individual2) [112] with ADMIXTOOLS [33] using the merged 1000 genomes dataset described above. The results were read into GNU R and plotted using the heatmap function (using the non-default parameters Colv=FALSE, scale='none') (Figure S4A). The results are summarized in Data S1, sheet 9.

Genetic structuring between burial locations

To investigate genetic structuring between the burial locations we have tested genetic clustering based on pairwise f_3-values between all ancient individuals. First we created distance matrix from pairwise f_3-values and plotted the values as an MDS plot using *cmdscale R* (Figure S4E). Since f_3-variation within different groups (excluding Cemetery 2 which was represented by one individual only) varied between sites (Figure S4E) we then used Kruskal-Wallis test to estimate levels of f_3-variation within the groups. The results indicated significant variation in within-group differences (chi-square = 16.851, df = 4, p value = 0.002066). The variation was higher than expected by chance (p = 0.04, tested over 100,000 permutations). Finally, we then tested for differentiation between groups by calculating mean similarities between members of two groups and using permutation test to check whether the observed similarities were lower than expected by chance (Data S1, sheet 4).

Rare allele sharing

Using outgroup f_3-statistics and PCA, we achieved a general overview of the affinities between the ancient Sigtunians and modern populations. These approaches are based on common alleles and tend to display long-term population structure. In order to look into more recent population history, we looked at the sharing of rare variants between the ancient individuals and modern groups [36]. We used vcftools [113] to extract SNP sites with minor allele counts from 1 to 10 from the European 1000 genomes populations FIN, GBR, IBS and TSI [109]. All of these populations have similar sample sizes of ~100 individuals. Then we used SAMtools [48] to identify all rare variant sites covered by at least one read and minimum mapping and base quality of 30 and counted the proportion of those sites which carried the minor allele from the modern populations. To estimate standard errors, we employed a block-jackknife procedure with a block size of 5000 SNPs (Figures S4B and S4C).

Functional SNPs

We have analyzed a number of functional SNPs in the seven medium coverage genomes from Sigtuna (84001, 84005, grt035, grt036, kal006, stg021 and urm160). We restrict the analyses to genomes with highest coverages to avoid listing samples with excessive amounts of missing data.

We have tested all seven ancient individuals at both LCTa (rs4988235) and LCTb (rs182549) loci responsible for lactase persistence. It has previously been suggested that the ability to digest milk was uncommon among early farmers and first increasing in frequency in European Iron Age [34]. In accordance with that expectation, we found that all but one of the tested individuals were lactase persistent while one (kal006) may have been lactose intolerant. However, the latter individual did not present any reads spanning position LCTa (rs4988235) and therefore the phenotypic state of kal006 should be treated with caution. In short, as could be expected form an average present-day north-European population, most of inhabitants in early medieval Sigtuna were carriers of lactase persistence alleles, meaning they were able to digest milk as adults.

All individuals showed high variability at AGT gene associated with increased risk of hypertension with three out of seven individuals carrying alleles minimizing the risk (84001, grt035 and urm160). Most individuals were carriers of KITLG (rs4590952) allele associated with protective UV tanning response and increased risk of testicular cancer [114]. Simultaneously, many carried non-risk associated alleles for prostate and ovarian cancers CYP3A4 (rs2740574). All individuals were carriers of risk associated G/C allele at CYP3A5 (rs776746) promoting the tendency to build up higher blood levels of the immunosuppressive drug called tacrolimus.

None of the individuals was a carrier of C/G allele at EDAR gene (rs3827760) associated with straighter and thicker hair as well as increased probability of having shovel-shaped teeth. This variant is often used as evidence of Asian admixture or ancestry since the C/G variant is prevalent in East Asians. However, another locus, which can be used in similar manner, ABCC1 (rs17822931), associated with ear-wax thickness, strength of body odor and production of colostrum, showed high variability among tested individuals with most of the individuals carrying at least one C variant, more common in Europeans and Africans, while kal006 may have been a (T;T) homozygote, commonly found in East Asians.

None of the individuals was a carrier of risk A allele at the ALDH2 (rs671) suggesting absence of the Asian flush and reduced hangovers. Moreover, all were carriers of C/G variants at the ADH1Bb gene (rs1229984) associated with increased alcohol consumption leading to increased risk of alcohol addiction.

Finally, we tested all seven individuals for presence of alleles associated with hereditary haemochromatosis. The genetic disease caused by mutations in HFE gene resulting in iron overload [115] was earlier suggested to have been associated with either Viking or Celtic expansions [9, 116].

Pigmentation

We attempted to reconstruct the physical appearance of the seven individuals from Sigtuna by predicting eye and hair color. The probability for certain eye and hair shade for each of the seven ancient individual was computed using the HIrisPlex

(http://hirisplex.erasmusmc.nl/) online tool [53–55]. The system was designed for DNA-based phenotype prediction for forensic purposes. The allelic status of SNPs associated with the predictions, as well as probabilities computed by HIrisPlex are listed in Data S1, sheet 5 and sheet 6. Since HERC2 gene rs12913832 is essential for eye-color prediction inputs missing value at that position do not result in phenotype prediction. Hence, no iris pigmentation information is available for individual kal006. Interestingly, all six remaining individuals were derived homozygotes at rs12913832, a variant associated with blue eye-color phenotype [117, 118]. Almost none of the individuals were carriers of variants typically associated with red hair (rs1805006, rs1805007, rs1805008, rs1805009), but they were all predicted to have had blond hair color of varying shades (Data S1, sheet 5). Based on the genetic variants at the positions rs16891982 (in the SLC45A2 gene) and rs1426654 (SLC24A5) SNPs (Data S1, sheet 5) all tested individuals are predicted to have been light-skinned [119–121].

DATA AND SOFTWARE AVAILABILITY

The accession numbers for the genome data reported in this paper are ENA: ERS2540882–ERS2540904.